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In re Application of:	)	Art Unit: 1724
Richard SMITH	)	Examiner: C. BARRY
Serial No.: 09/66:,507	)	Confirmation No. 2262
Filed: September :4, 2000	)	Washington D.C.
For: SMALL-SCALE HYDROGEN-OXIDIZING-DENITRIFYING BIOREACTOR	)	

DECLARATION UNDER 37 CFR 1.131

I, Richard L. Smith, do hereby declare that I am the sole inventor of the above-captioned application.

Attached hereto are true copies of entries to my HOD Bioreactor Notebook #3, which entries were made prior to January ., 2000, the effective filing date of Rittmann et al., U.S. Patent No. 6.387,262. All work described in this declaration was conducted at the US Geological Survey in Boulder, Colorado.

It should be noted on page 80 that the notebook describes the four components of the apparatus as claimed, namely:

a. autotrophic, hydrogen-oxidizing denitrifying bacteria;

b. a water electrolysis unit that provides a continual supply of oxygen-free hydrogen;

c. a flow-though bioreactor that contains the HOD bacteria and is designed to maximize their ability to remove nitrate in the presence of hydrogen; and

d. a sand filtration unit to remove unwanted microbial biomass from the treated water.

Additionally, opposite page 82 of the notebook pages is a figure of the hydrogen generator and denitrifying bioreactor and sand filter.

It is clear that this invention had been reduced to practice prior to January 1, 2000, because, as stated on page 84 of the notebook, optimum residence time had been determined.

I hereby further declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 81 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issued thereon:

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D. Explanation of a specific embodiment of the invention.

1. Theory of operation

The device described herein consists of 4 principle components. These are: 1) autotrophic, hydrogen-oxidizing denitrifying bacteria isolated from subsurface environments; 2) a low-cost water electrolysis unit that provides a continual supply of oxygen-free hydrogen; 3) a flow-through bioreactor that contains the HOD bacteria and is designed to maximize their ability to remove nitrate in the presence of hydrogen; and 4) a sand filtration unit to remove unwanted microbial biomass from the treated water.

Hydrogen-oxidizing denitrifying (HOD) bacteria obtain their energy by oxidizing hydrogen gas and coupling that to nitrate reduction (Figure 1). They occupy a unique ecological niche, one in which there is little competition from other microorganisms. The end products of the HOD process are water and [illegible] drinking water supply, as is hydrogen. In addition, many of the HOD bacteria in groundwater are autotrophs (Smith et al., 94). That means that they use carbon dioxide as a carbon source for growth; they have no additional carbon requirements. Because carbon dioxide is present in natural waters as carbonate, these bacteria can be used to remove nitrate in a water supply simply by adding hydrogen gas. Such a treatment is very selective for HOD bacteria, excluding all other types of microorganisms that could not grow under such conditions. The HOD bacteria can also utilize hydrogen and respire aerobically. This trait is very useful in a nitrate removal bioreactor because oxygen inhibits denitrification. Thus, oxygen must first be removed from any water supply before denitrification can commence within the reactor. However, the same HOD culture can effect both oxygen and nitrate removal, as long as an adequate supply of hydrogen is available.

Hydrogen gas has a low solubility in water. This low solubility requires that an excess of hydrogen is always available to remove the quantities of nitrate found in many contaminated water supplies. Hydrogen that is not utilized by HOD bacteria in the treatment process can be easily removed from the water by aeration. Hydrogen can be generated via electrolysis of water, which produces hydrogen gas at the anode and oxygen gas at the cathode at a molar stoichiometry of 2:1. The amount of hydrogen produced is dependent upon the voltage applied to the electrodes and the electrolyte concentration.

Flow-through bioreactors are designed to provide a fixed stationary support for an attached microbial biofilm. The biofilm contacts or is immersed in a flowing aqueous stream and removes or alters the chemical composition of the water via the activity of the attached microorganisms. In some cases, nutrients or substrates for the microorganisms need to be added to the bioreactor. If the substrate is a gas (such as hydrogen), counter current flow of the gas and the

$$H_2 + NO_3^- \xrightarrow{\text{HOD BACTERIA}} H_2O + N_2$$

$$CO_2 \longrightarrow \text{CELL CARBON}$$

FIGURE 1. HYDROGEN COUPLED DENITRIFICATION

water is advantageous to increase the availability of the gas to the microorganisms. This can also serve as a mechanism to strip other unwanted gases, such as oxygen, out of solution.

2. Detailed description of invention.

An embodiment of the present invention to remove nitrate from a small-scale water supply using the HOD reaction is shown in Figures 2 and 3 and consists of the 4 components listed in the above section. The numbers within the text refer to the numbered items in the figures.

Component 1. HOD Bacteria.

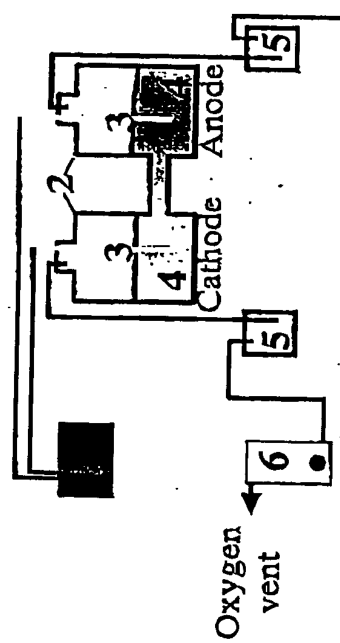
Pure cultures of autotrophic, hydrogen-oxidizing, denitrifying (HOD) bacteria are used as the reactive agents in the flow-through bioreactor used in this invention. The bacteria have been isolated from nitrate-containing groundwater environments. This makes them ideal for such a treatment system because an aquifer is characterized by water flowing through a porous medium, which is identical to the function of the bioreactor. These microorganisms require no organic carbon for growth, only hydrogen, nitrate, and carbon dioxide.

Several strains of HOD bacteria have been isolated from groundwater and partially characterized. Strain HOD 5 is used in the present embodiment of this invention. This strain is partially described in Smith et al (1994). The bacterium is a gram negative, motile rod, that can grow on hydrogen using either oxygen or nitrate as electron acceptors. It can also grow aerobically on nutrient broth, acetate, pyruvate, lactate, succinate and glutamate. Phylogenetic analysis of the full sequence of the 16S rRNA gene reveals that HOD 5 belongs to the beta subclass of the proteobacteria and is most closely related to purple, non-sulfur, phototrophic bacteria, particularly Rhodocyclus species. For the bioreactor, a pure culture of HOD 5 is grown in batch culture on hydrogen and nitrate using HOD medium (Smith et al. 1994). Following development of turbidity, the culture is transferred to the bioreactor column (see below; component 3) which has been filled with HOD medium. The culture is grown statically in the bioreactor, with hydrogen flowing, for 2-3 days before the water supply is turned on.

Component 2. Hydrogen Generator.

Hydrogen gas is produced by hydrolysis of water in a dual-chamber, glass reservoir (2). The two chambers are each sealed with a pressure-tight screw top cap that is penetrated with a platinum wire electrode (3). The chambers are connected via hollow glass tubing and contain 4 N sodium hydroxide. A 12 volt 2 amp DC electrical potential is continuously applied to the electrodes using a commercial automobile battery charger (1). Oxygen gas is produced in the cathode chamber, and is channeled via metal tubing through a sodium hydroxide trap (5) to an adjustable gas flow controller (6). Hydrogen gas is produced in the anode chamber and is channeled through a sodium hydroxide trap (5), a check valve (7) to prevent back flow, and into the bioreactor (8-10). Internal pressure

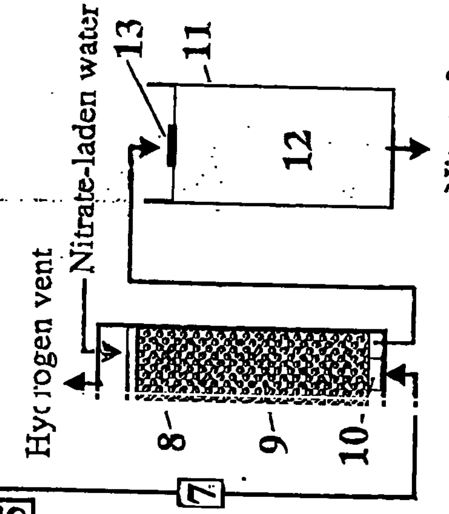
Fig 2. Hydrogen Generator



Component List

1. 12 volt, 2 amp, DC power supply
2. Glass containers, with pressure tight screw top lids, connected via side arm tubing
3. Platinum wire electrodes
4. 4 N Sodium hydroxide
5. Sodium hydroxide trap
6. Adjustable flow meter
7. Check valve
8. Flow-through plastic pipe, with endcaps
9. Sorted pea-gravel, 2-4 mm
10. Airstone
11. Plastic pipe with endcap on bottom
12. Washed sand
13. Water distribution block

Fig 3. Denitrifying Bioreactor and Sand Filter



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within the 2 chambers of the hydrogen generator is balanced using the adjustable flow controller.

Component 3. Flow-Through Bioreactor.

The flow-through bioreactor (8-10) is constructed from plastic pipe and fitted with sealed endcaps. The bioreactor is filled with a coarse porous medium (9) such as washed pea gravel (2-4 mm diameter) or plastic or glass beads, which serve as solid surfaces to support biofilm formation by the HOD bacteria. Nitrate-laden water is pumped into the top of the reactor, travels downward through the porous medium where it contacts the microbial biofilm, and exits out the bottom of the bioreactor nitrate-free. The water level within the bioreactor is controlled by the height of the exit tube.

Hydrogen gas enters the bioreactor via an airstone (10) in the bottom. Hydrogen bubbles travel upward, countercurrent to water flow, and are vented out the top endcap. In addition to serving as substrate for the HOD bacteria, the hydrogen bubbles strip oxygen from the influent water and nitrogen gas from water within the reactor that is produced via the denitrification reaction. The headspace volume in the bioreactor is designed not to exceed 1-5 % of the total volume of the bioreactor to minimize the amount of hydrogen gas present within the system.

Component 4. Sand Filtration Unit.

The nitrate-free water exiting the bioreactor then percolates via gravity flow through a sand filtration unit (11-13). This unit is constructed with plastic pipe that is fitted with a bottom endcap. The unit is filled with a bottom layer of pea gravel 4-6 inches thick, and overlain with clean, coarse- to medium-grained sand (12). On top of the sand column is a block (13) to evenly distribute the input water over the surface of the sand. The overall height of the sand filter unit is approximately equivalent to the height of the water column within the bioreactor. In the sand filter, the water is aerated and filtered to remove suspended microorganisms from the bioreactor effluent. The top layer of sand within the infiltration unit is periodically removed and replaced with clean sand. Water exits the sand filter unit via a tube inserted in the bottom endcap.

3. Preferred and extreme ranges of conditions.

Optimum hydraulic residence time for the bioreactor for a nitrate concentration of 2 mM (28 mg/L N) is 1.5-2 hours at a temperature of 25 °C. The bioreactor can effectively remove nitrate concentrations from 0.7-20 mM (10-280 mg/L N) in a pH range of 6-9.

Rocky	pH
IN	8.38
OUT	9.61
ALT OUT	9.61 -
SAND	8.94

11:45 am

O_2 height: 3 cm

H_2 still flowing

O_2 rate 1mL/13sec

Worked on Data

Made new Batch of Well H_2O + Nitrate

pH = 8.08